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REVIEW

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T cell vaccination: clinical application in autoimmune diseases

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Abstract T cell responses to myelin basic protein (MBP) are implicated to play an important role in the pathogenesis of multiple sclerosis (MS). These MBP autoreactive T cells are found to undergo in vivo activation and clonal expansion in patients with MS. They accumulate in the brain compartment and may reside in the brain lesions of patients with MS. As MBP-reactive T cells potentially hold a central position in initiation and perpetuation of the brain inflammation, specific immune therapies designed to deplete them may improve the clinical course of the disease. We review here the recent application of T cell vaccination in patients with MS to deplete circulating MBP-reactive T cells. The results of our phase I clinical trial indicate that T cell vaccination with inactivated MBP autoreactive T cells induces specific regulatory T cell network of the host immune system to deplete circulating MBP-reactive T cells in a clonotype-specific fashion. The immunity induced by T cell vaccination is clonotype specific and long-lasting. Our longitudinal clinical evaluation further suggests a moderately lower rate of clinical exacerbation, disability score, and brain lesions (measured by magnetic resonance imaging) in vaccinated patients than in matched controls. Our study should encourage further investigation on the treatment efficacy of T cell vaccination and further improvement for its clinical administration in other human autoimmune diseases. This review discusses the immune regulation and therapeutic administration of T cell vaccination in human autoimmune diseases, exemplified by our recent T cell vaccination trial in MS.

Key words Autoimmune disease · T cell vaccination · T cell clone · Multiple sclerosis · Myelin basic protein

Abbreviations *EAE* Experimental autoimmune encephalomyelitis · *EDSS* Expanded Disability Status Score · *MBP* Myelin basic protein · *MRI* Magnetic resonance imaging · *MS* Multiple sclerosis · *PHA* Phytohemagglutinin · *TCR* T cell receptor

Introduction

Autoreactive T cells recognizing a variety of self antigens represent part of the normal T cell repertoire and naturally circulate in the periphery without causing autoimmune disease [1]. The critical transition from autoreactivity, a normal physiological state, to autoimmune pathology is potentially determined by the interplay between activation and clonal expansion of autoreactive T cells and the functioning of regulatory networks that keep them in check. These autoreactive T cells are found to undergo in vivo activation and clonal expansion in various organ-specific human autoimmune diseases. They migrate to and accumulate at the site of pathology and hold a central position in initiation and perpetuation of local inflammation. Recent advances in our understanding of the structural and functional properties of autoreactive T cells, in particular the regulation of these autoreactive T cells, have prompted clinical trials designed specifically to suppress potentially pathological autoreactive T cells in human autoimmune conditions with some clinical benefits [2–4].

The mechanism by which autoreactive T cells are regulated in vivo remains unclear. There is evidence suggesting that one of the regulatory mechanisms involves the clonotypic regulatory network. These regulatory T cells, termed anticlonotypic T cells, are thought to regulate autoreactive T cells in recognition of clonotypic determinants within the T cell receptor (TCR) [5]. Together with their

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target autoreactive T cells these specialized regulatory T cells are proposed to constitute part of the internal image of the immune system. This concept has led to the paradigm of T cell vaccination in which attenuated autoreactive T cells are used to immunize and protect experimental animals against autoimmune diseases [6]. T cell vaccination has now been advanced to treat human autoimmune diseases in a clinical setting, as shown in our recent clinical trial. The recent studies have begun to shed some light on the functional properties of the immune regulatory network that operates in vivo and can be stimulated by T cell vaccination to constrain potentially pathological autoreactive T cells. More importantly, the clinical results of T cell vaccination suggest potential clinical benefits in treated multiple sclerosis (MS) patients and encourage further investigation on the treatment efficacy and further improvement for broad clinical application.

Pathological role of autoreactive T cells in multiple sclerosis

Although the cause of MS remains unknown, increasing evidence suggests that autoimmune mechanism directed at myelin tissue of the central nervous system plays an important role in the pathogenesis of MS [7]. The encephalitogenic potential of T cell reactivity to myelin components, such as myelin basic protein (MBP) and proteolipid protein, can be demonstrated in experimental autoimmune encephalomyelitis (EAE), an animal model for MS [8]. T cells recognizing encephalitogenic epitopes on MBP or proteolipid protein are the direct mediator of the CNS inflammation seen in EAE [9]. Activation and clonal expansion of MBP-specific T cells is the hallmark of the pathological properties of these encephalitogenic T cells, representing a functional requisite for their ability to induce EAE [10]. Activation upon a challenge with MBP or proteolipid protein induces a different functional state and a homing pattern into the central nervous system to which the myelin destruction is confined. As a result, the TCR V gene repertoire of peripheral MBP-specific T cells shifts to a restricted V gene pattern characteristic for the clonally expanded encephalitogenic T cells [10]. These studies have yielded some valuable information that may bear potential relevance for the role of myelin autoreactive T cells in the autoimmune mechanism involved in MS. Furthermore, the restricted TCR V gene usage of MBP-reactive T cells found in EAE provides a suitable target for various specific therapeutic interventions recently designed to prevent and treat EAE [11–13]. These studies have shown remarkable clinical effectiveness and generated new enthusiasm that human autoimmune diseases may be amenable to selective treatment in a similar fashion.

It should be noted that although MBP-reactive T cells are present in blood circulation of both patients with MS and healthy individuals, they display different functional and structural characteristics which may be related to their potential pathological role in MS. These MBP-reactive T cells are found to undergo in vivo activation and accumulate in the brain compartment of patients with MS, as opposed to healthy individuals [14–16]. Direct analysis of TCR V genes and the CDR3 region sequences in postmortem MS plaques has revealed clonal expansion among lesion-derived T cells that share a sequence homology with MBP-reactive T cells [17]. Consistent with these findings are clonal expansion of circulating MBP-reactive T cells in patients with MS [18, 19], suggesting that the disease-related processes may drive certain MBP-reactive T cell clones to proliferation/expansion. Together these data lend strong support for the potential pathological role of T cell response to MBP in MS.

Autoreactive T cells as therapeutic target

An immunotherapy can be designed based on the mechanisms underlying the pathogenesis of the disease regarding the pathogenic autoantigens and autoreactive T cells responsible for the autoimmune pathology. These studies are largely inspired and guided by experimental animal models. The subunit of the T cells that distinguishes the pathogenic T cells from other unrelated T cells is the TCR. The TCR seems to be the most appropriate target structure in designing an effective and specific immunotherapy. To be successful an obvious requirement for targeting the TCR is a restricted TCR repertoire of a given pathogenic T cell population. This condition seems to be met in EAE where activated encephalitogenic T cells are the direct cause of the disease and their TCR repertoire towards MBP is rather limited with respect to a restricted epitope recognition and a biased V gene usage. Hence the limited TCR repertoire provides suitable molecular targets for specific therapeutic interventions. Various TCR-based strategies have been developed to target at TCR V gene products or other attacking points within the TCR complex charateristic for the encephalitogenic T cells (for a review see [1]).

As a disappointment to the original expectation, however, further studies on human autoreactive T cells in several autoimmune diseases have revealed that the TCR repertoire of human autoreactive T cells is rather heterogeneous. For example in MS, our study and a number of other independent studies indicate that MBP-reactive T cells use diverse TCR repertoire in recognition of the immunodominant regions of MBP, the 84–102 and 143–168 regions [20–24]. Unlike in rodent EAE, the TCR V gene repertoire of MBP autoreactive T cells varies among patients with MS. They use a broad spectrum of Vβ genes in response to MBP [20–24]. Evidence accumulated so far argues against a potential association of a TCR Vα and Vβ combination(s) with MS since no common TCR V gene pattern(s) could be accounted for the disease association. Thus the heterogeneous expression of TCR V gene products among a general MS population would considerably perplex the attempts to develop an immunotherapy directed at a "common" variable region(s) of the TCR. A treatment agent (e.g., a mono-

clonal antibody or a TCR peptide) designed to target at certain TCR V gene product(s) may be useful in one patient but is not suited for another, which significantly hampers its clinical usefulness.

However, the TCR V gene repertoire is not totally random, and it appears to be restricted in given MS patients. Most recent studies have revealed that the limited Vβ gene usage in a given MS individual rather represents clonal expansion of MBP-specific T cells in blood circulation, as evidenced by their sharing of unique V-D-J and V-J junctional DNA sequence patterns [18, 19]. The activation and clonal expansion of autoreactive T cells in MS are in accordance with recent studies that demonstrate the oligoclonal nature of T cells confined to the cerebrospinal fluid and the postmorterm brain lesions in patients with MS [14–16]. Thus, as clonal expansion of limited MBP-specific T cell population is a rather profound feature in MS, their restricted TCR repertoire provides a uniform target structure in a given patient even though it varies between individuals. Similar to the TCR repertoire shift associated with acute EAE in rodents, in some patients with MS, these clonally expanded T cells often account for more than 60–80% of all MBP-specific T cells in a given individual with MS. Thus these T cells that undergo in vivo clonal expansion and bear a uniform clonotypic determinant(s) have an obvious therapeutic potential for immune intervention.

T cell vaccination: basic principle and laboratory findings

As pathogenic autoreactive T cells are viewed as pathogens in T cell-mediated autoimmune diseases, they can be used, when rendered attenuated by irradiation or chemical treatment, as vaccines to prevent and treat the diseases which they are able to induce [6]. The principle of T cell vaccination is similar to traditional microbial vaccination against infectious agents. Pioneering work by Irun Cohen and coworkers has provided experimental evidence that administration of attenuated autoreactive T cells as vaccines induces the regulatory networks to specifically suppress the eliciting autoreactive T cells [25]. T cell vaccination is effective in preventing and treating many experimental autoimmune diseases, including EAE, experimental autoimmune uveitis, experimental diabetes model, and adjuvant arthritis. The protective effect is long-lasting and specific since the autoreactive T cells used for vaccination only protect against the disease that they are able to induce [25].

The mechanism underlying T cell vaccination is not completely understood but is thought to involve clonotypic network regulation directed at clonotypic determinants of a target TCR. The data accumulated so far from the animal studies indicate that the anticlonotypic T cell responses induced by T cell vaccination do not seem to recognize TCR framework regions whose sequences are germ-line encoded and are tolerant by the immune system. Rather, the target epitope(s) most likely lie within the hypervariable regions, including CDR3 or less variable CDR2, as predicted by their immunogenic property and sequence diversity [26]. There is experimental evidence from recent investigations involving immunization with various TCR peptides that both the CDR2 and the CDR3 regions are likely to trigger the clonotypic interactions [11, 12]. Evidence supporting this possibility comes from several observations. For example, a clonotypic regulatory T cell response can be induced by immunizing rats with a synthetic peptide of the Vβ CDR2 region characteristic for encephalitogenic T cells to prevent EAE. These anticlonotypic T cells are the major cellular component of the protective mechanism and are capable of conferring a specific protection to naive rats by adoptive transfer [11, 12]. Similarly in humans TCR peptides corresponding to both CDR2 and CDR3 regions are found to induce $CD4^+$ regulatory T cells in vitro [27]. Furthermore, other regulatory T cells induced by T cell vaccination may also contribute to the protection by interacting with cellular markers other than the TCR clonotypes, such as the regulatory T cells so-called antiergotypic T cells that respond not to the TCR but to a cell marker associated with their state of activation [28]. In this context, T cell vaccination offers a unique in vivo setting in which the clonotypic network, together with other regulatory mechanism, is stimulated to interact selectively with native target epitope(s) of an immunizing T cell to down-regulate the autoreactive T cells selected for vaccination.

Preliminary clinical studies on T cell vaccination in humans

An initial clinical trial was carried out in four patients with MS by Hafler and coworkers. Patients were inoculated with formadehyde-fixed autologous T cell clones isolated by stimulation with phytohemagglutinin (PHA) from cerebrospinal fluid. The T cell clones used for vaccination were chosen based upon their phenotype ($CD4^+$), growth characteristics, and the expression of dominant rearranged TCR genes which represent the oligoclonal T cells circulating in the cerebrospinal fluid. The study revealed some interesting immunological findings regarding a general inhibition on T cell stimulation via the CD2 pathway and increases in autologous mixed lymphocyte responses after vaccination [29]. Since the antigen specificity of these T cells was not defined at the time, and the generation of T cell clones was not selective (polyclonal stimulation by PHA), it was not clear whether obtained T cell clones represent pathologically relevant autoimmune T cells, and whether vaccination with these cerebrospinal fluid-derived T cells down-regulated the relevant autoreactive T cell population. Two other clinical studies in rheumatoid arthritis were have been reported by De Vries and associates. In one study synovial T cells derived from 13 patients with rheumatoid arthritis were activated polyclonally by a mitogenic anti-CD3 antibody and used for vaccination, based on the assumption that these heterogeneous cell preparations contain pathogenic T cells activated by a

putative autoantigen(s) within the synovial compartment [30]. In another study T cell lines reactive to nickel were used for vaccination in nickel-sensitized donors to assess the immune responses to the immunizing T cells [31]. In neither case, however, were significant immune responses observed as compared to the prevaccination values with respect to T cell response to the immunizing T cells and delayed-type hypersensitivity reaction. Other investigators, however, have demonstrated T cell response to the immunizing T cells after T cell vaccination in nonhuman primates and human subjects [32]. In these studies the clinical parameters were not assessed systematically. T cells used for vaccination were heterogeneous and were not defined for antigen specificity. It is conceivable that in these cases the immunizing T cells carried highly diverse T cell receptors which are unlikely to direct the host immune system specifically to the pathologically relevant autoimmune T cells. In addition, the different immunization protocols used in these studies may account for the discrepancies in the observed immune responses. Nevertheless, these clinical studies demonstrated that toxicity related to the subcutaneous vaccination of attenuated autologous T cells does not seem to be a major concern. Administration of inactivated autologous T cells is well tolerated in humans and causes no adverse effect.

On the other hand, although TCR peptides corresponding to CDR2 and CDR3 have been shown to be useful in treating EAE in rodents [11, 12], these TCR peptides are selected and synthesized according to their presumed immunogenic property and stimulate only CD4+ regulatory T cells. It is unclear whether these selected TCR sequences correspond to the native sequence region(s) that is naturally recognized by the clonotypic network in vivo to down-regulate these autoreactive T cells, and whether this CD4+ MHC class II restricted pathway represents the most effective mechanism by which the immune system regulates autoreactive T cells. Some of these studies have recently been advanced to human trials, such as in MS. Vandenbark and coworkers immunized MS patients with two synthetic CDR2 peptides corresponding to TCR Vβ 5.2 and Vβ 6.1 in a phase I clinical trial [4, 33]. The study revealed some interesting findings that the immune response induced by the TCR peptides seemed to affect the frequency of circulating MBP-reactive T cells in treated patients [4, 33]. However, as discussed above and recently concluded at an international workshop [34], MBP autoreactive T cells display a heterogeneous pattern of the TCR Vβ gene usage, even though the V gene usage is considerably restricted in a given individual as a result of in vivo clonal expansion. Thus, the TCR peptides must be designed individually and tailor-made to treat a given patient, depending upon the individualized Vβ gene rearrangement of MBP-reactive T cells.

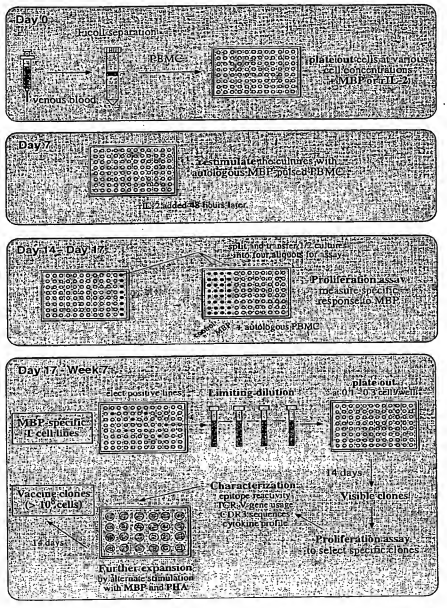
Technical feasibility and protocols

With the T cell culture and cloning techniques developed in our laboratory it is now technically feasible to generate MBP-reactive T cell clones in therapeutic amounts required for T cell vaccination. MBP-reactive T cell clones are prepared using a two-stage process (Fig. 1). In the first stage multiple independent MBP-reactive T cell lines are generated from a blood sample. This is carried out in a micro-culture system, which allows an unbiased in vitro expansion of individual clones of the entire MBP-specific T cell repertoire. When combined with serial predetermined plating cell concentrations, the precursor frequency of circulating MBP-reactive T cells can be estimated by the Poisson statistics [35]. In the second stage the resulting MBP-reactive T cell lines are further cloned by a "single-cell" cloning procedure under classic limiting dilution conditions using PHA and autologous peripheral blood mononuclear cells as a source of accessory cells. When a stable clone is established, a two-round stimulation can yield a cell expansion factor ranging from 50 to 100.

Based on the results of the preliminary clinical studies [29–32], toxicity related to the subcutaneous vaccination of attenuated autologous T cells does not seem to be a major concern. Administration of various forms of autol-

Fig. 1 Precursor frequency analysis, generation, and cloning of MBP-reactive T cell lines for immunization. MBP-reactive T cell clones are generated in a two-stage procedure, from day 0 to week 5. *In the first stage*, peripheral blood mononuclear cells (*PBMC*) are plated out at 200,000 cells/well, 100,000 cells/well and 50,000 cells/well in the presence of MBP or a low concentration of recombinant interleukin-2 (*rIL-2*; to stimulate activated MBP-reactive T cells; see [14]). After 7 days the cultures are refed with 100,000/well irradiated autologous PBMC pulsed with MBP as a source of antigen-presenting cells (*APC*), and rIL-2 is added 48 h later to supplement the T cell growth. This restimulation allows further expansion of MBP-reactive T cell cultures and, in particular, is essential for selective expansion of activated MBP-reactive T cells from cultures primarily stimulated with IL-2. At day 14 half of each culture is split into four aliquots and tested, in duplicates, for specific proliferation to MBP or tetanus toxoid (used as a control antigen) in a proliferation assay (thymidine incorporation). A T cell line is defined as specific when CPM of the wells containing MBP-pulsed APC/CPM of control wells exceeded 3 and Δ CPM >1000. The frequency of MBP-reactive T cells can be calculated according to the Poisson statistics (see [35]). Briefly, by scoring the number of positive cultures, a frequency of positive wells is obtained at each cell concentration. Estimation of the frequency of MBP-specific T cells is then carried out by applying the Poisson Formula in which $Fr=(u^r/r!)\times e^{-u}$, where Fr is the probability of obtaining r specific T cells in a well when the number of PBMC per well is u at a given concentration. The fraction of negative wells is given by $F_0=e^{-u}$. When $u=1$, $F_0=0.37$. Therefore, theoretically, when the average number of responding T cells per well is one, 37% of the wells will be scored as negative. Extrapolation to this point in limiting dilution gives a number of cells, the reciprocal of which represents the frequency of MBP-specific T cells in question. *In the second stage*, the resulting MBP-reactive T cell lines are subsequently cloned under limiting dilution condition. Cells of each T cell line are seeded at 0.1–0.3 cell/well and stimulated with PHA-protein in the presence of irradiated autologous PBMC. Cultures are fed with fresh IL-2 containing medium every 4 days. Usually after 14 days growth-positive wells (less than 10% in all cases) become visible and can be tested for their specific reactivity to MBP in the above proliferation assays. The clones are then subject to further characterization regarding their epitope specificity and TCR V gene analysis. Further expansion of the clones are carried out by two cycles of alternate stimulation with MBP and PHA, which typically yield greater than 10^8 vaccine T cells

ogous T cells is well tolerated in human subjects and produces no adverse effects, as evidenced in these pilot human trials. However, there are several factors that might influence the way the immune system interacts with the immunizing T cells, which may affect the clinical effectiveness of T cell vaccination. First, the selection of autoreactive T cell clones has an obvious importance in focusing the immune attack at pathogenic T cells to spare other unrelated T cells. It is a rather cumbersome task to select relevant autoreactive T cell clones for vaccination, given that the TCR repertoire is relatively heterogeneous and varies among individual patients. In this regard recent TCR repertoire studies, in particular the analysis of the CDR3 region sequences, have provided some clues to select a relevant T cell clone(s) for vaccination. As discussed above, MBP-reactive T cells undergo in vivo clonal expansion in MS, resulting in a shift of the TCR repertoire dominated by the clonally expanded population(s). In vivo clonal expansion is rather instructive for their pathological relevance since the clonal expansion of myelin autoreactive T cells is presumably driven by and related to the disease process. Based on



the observation that clonally expanded MBP-reactive T cell population often represents 60–80% of the MBP-specific T cell repertoire in a given MS patient, it is tempting to propose that specific depletion of a clonally expanded population(s) would eradicate the major T cell response to MBP. In our clinical trial multiple MBP-reactive T cell clones generated from each patient were characterized extensively and sequenced for their CDR3 sequence patterns to identify in vivo expanded clones for vaccination. In a few cases where the TCR repertoire of MBP-reactive T cells is so heterogeneous, all clones, preferentially those reactive to the immunodominant epitopes (the 84–102 and the 143–168 regions), were pooled to make a "cocktail vaccine" preparation.

In addition to the selection of relevant vaccine clones, the way in which the T cells are activated in vitro may also affect their effectiveness in T cell vaccination. Activated vaccine clones are more effective in the induction of a protective response than resting vaccine clones, presumably owing to the expression of activation-associated T cell surface molecules. Activation of vaccine T cells by both antigenic and mitogenic stimulation in the presence of antigen-presenting/accessory cells has been shown to effectively induce the protective response in rodent EAE. The accessory cells seem to deliver additional signals required to sufficiently stimulate the host immune system. This is suggested by the inability of vaccine T cells activated with interleukin-2 alone, in the absence of accessory cells, to induce the protective effect (I. Cohen, personal communication). Furthermore, the mode of attenuation may also influence the immunogenicity of the vaccine clone in eliciting the immune responses. We used irradiation to attenuate the vaccine clones, based on our view that while irradiation effectively attenuates T cells, it preserves the major physiological features of the cell surface markers and the membrane stability. These unaltered features are important to render vaccine T cells recognizable in the same manner as they are seen by the immune system in vivo.

The dose factor is also critical to the induction of an adequate immune response. A high vaccine dose may not be necessarily helpful to induce a desired host immune response and may even be potentially hazardous [36]. There is recent experimental evidence suggesting that high doses of attenuated or dead pathogenic T cells can yield disease. In mouse spontaneous systemic lupus erythematosus model the administration of 2.5×10^5 irradiated T cells vaccinated mice against the disease, but a higher dose of 5×10^6 of the same attenuated T cells even enhanced the disease [37]. Under these considerations we used a minimal dose of attenuated T cells that is shown to be effective in experimental animal studies, as extrapolated on the basis of relative skin surface areas [2, 38, 39].

Recent T cell vaccination trial in multiple sclerosis

As discussed above, the TCR repertoire of MBP-reactive T cells is rather restricted in a "patient-dependent" fashion as the consequence of in vivo expansion of MBP-reactive T cells of a limited clonal origin(s). Hence, a specific immunotherapy may take advantage of T cell vaccination which utilizes the whole pathogenic autoreactive T cells and a natural preexisting regulatory network to specifically regulate pathogenic autoreactive T cells. In this scenario, the distinguishable cellular marker of "disease-related" autoreactive T cells is the TCR variable region(s) characteristic for the clonally expanded population in a given patient. The immune system is up-regulated to respond to the clonotypic determinants in association with other related cellular markers, in their naturally assembled form, to achieve an adequate regulatory response. T cell vaccination appears to merit its place in designing such a specific therapeutic strategy for human autoimmune pathologies. These considerations have provided the rationale for clinical trials using T cell vaccination in patients with MS.

In 1992 we initiated a phase I clinical trial in which patients with relapsing-remitting and chronic progressive MS were vaccinated with irradiated autologous MBP-reactive T cell clones. This limited phase I clinical trial was aimed to address some primary questions regarding the technical feasibility, toxicity, regulatory T cell responses, and potential changes in the frequency of circulating MBP-reactive T cells after vaccination. Can T cell vaccination be tolerated in human subjects? Is T cell vaccination able to induce a specific anticlonotypic response? And is the induced anticlonotypic response effective to suppress or deplete circulating MBP-reactive T cells in vaccinated patients? Disease activity including the frequency and severity of clinical exacerbation, neurological examination and the magnetic resonance imaging (MRI) of the brain lesions was also monitored to potentially correlate with the immunological changes in vaccinated patients. It was originally hoped that this clinical trial would set the stage to evaluate an optimal immunization protocol and methodology for the preparation and effective administration of the attenuated autologous T cells to achieve a desired immunological effect.

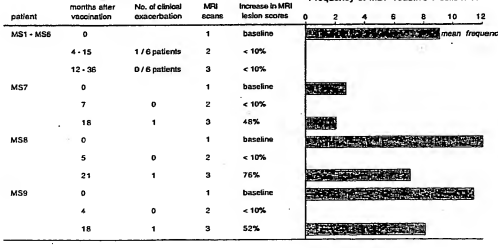
Selected MBP-reactive T cell clones (T cell vaccines) were first activated in vitro and irradiated subsequently to render them incapable of proliferation. Each recipient received a total of three subcutaneous injections of two to four vaccines clones (15×10^6 cells for each clone) at intervals of 2–4 months. Our experience has been that the procedure used for our vaccination trial is safe and technically feasible. Subcutaneous inoculations of the autologous vaccine clones are well tolerated and cause no adverse effects except skin redness at the injection site (usually after the second and third injection), which is reminiscent of a delayed-type hypersensitivity reaction. Our study revealed that vaccination with irradiated MBP-reactive T cells induces T cell responses that coincide reciprocally with a progressive decrease in the frequency of circulating MBP-reactive T cells in all vaccinated patients [2]. After three vaccinations no circulating MBP-reactive T cells were detected in the recipients, suggesting a depletion of the autoreactive T cells. Both

the anticlonotypic response (for their specific recognition of the immunizing clones but not autologous T cells with other antigen specificity) and the decline of MBP-reactive T cells were marked by a boosting effect with each vaccination. Furthermore, the depletion of circulating MBP-reactive T cells in vaccinated patients seems to be an antigen-specific event. In contrast to the disappearance of MBP-reactive T cells, the frequency of T cells specific for tetanus-toxoid, a recall antigen, did not change over the same period of time [2]. The in vivo depletion of MBP-reactive T cells may be associated with the direct effect of anticlonotypic T cells as the $CD8^+$ cytolytic anticlonotypic T cell lines isolated from the vaccinated patients specifically lyse the autologous vaccine clones [2]. Furthermore, our study suggests that the cytolytic anticlonotypic T cell response represents the major immune response induced by T cell vaccination and is pre-existing at a rather low frequency in MS patients prior to to vaccination. The cytolytic anticlonotypic response is boosted by each inoculation and their frequency mounts typically to a tenfold increase after the second and the third vaccination [2, 40]. This study has confirmed for the first time in a clinical setting that T cell vaccination can be used to boost clonotypic regulatory T cells in depleting pathologically relevant autoreactive T cells.

To further investigate whether depletion of MBP-reactive T cells in vaccinated patients correlates with clinical improvement, patients were monitored over a period of two to three years for various clinical parameters, including exacerbation rate, Expanded Disability Status Score (EDSS) and quantitative changes in brain lesions as detected by MRI. Each patient was paired with a control MS subject selected before vaccination. These control MS subjects were matched with the treated patients for age, gender, clinical characteristics (relapsing-remitting or chronic-progressive MS) and the disease duration. Our study demonstrates that as a group the five patients with relapsing-remitting characteristic exhibited a total of three exacerbations during 2–3 years after vaccination as compared to 16 relapses recorded during a 2-year period prior to vaccination [41]. In five paired control MS subjects the number of relapses was reduced from 12 to 9 during the same period of time. Consistent with the reduced rate of exacerbation are the relatively stabilized neurological function scores in the relapsing-remitting patients, as shown by a slower evolution in the clinical course and a net reduction of the end EDSS by 0.5–2.0 points in some patients [41]. In contrast, EDSS recorded in paired control subjects showed a progressive increase over the same period of time, which reflects the natural course of the disease within this group of patients. In three patients with chronic progressive MS, however, vaccination did not seem to alter the clinical course, and their EDSS advanced over time at a similar rate and with the same pattern as that of paired control subjects.

Throughout the clinical trial three MRI scans (heavily T2-weighted images) were taken for each vaccinated patient, before vaccination (baseline), shortly after vaccina-

Fig. 2 The changes in the frequency of circulating MBP-reactive T cells before and after T cell vaccination in correlation with rate of clinical exacerbation and the MRI brain lesion scores. The frequency analysis was performed by plating out peripheral blood mononuclear cells (*PBMC*) the presence of MBP at three predetermined concentrations. A T cell culture was defined as specific for MBP when counts per minute (cpm) of the wells containing MBP-pulsed antigen-presenting cells/cpm of control wells (in medium alone) exceeded 3 and Δcpm>1000. The frequency was calculated by the Poisson statistics when the frequency distribution followed the "single-hit" rule (see Fig. 1 legend). In cases of "multiple-hit" distribution the frequency was estimated by dividing the number of specific cultures by the total amount of PBMC plated at an optimal concentration that yielded the highest number of specific wells. For each individual the same method of calculation was used consistently to compare the frequency changes over time. Each data point represents the mean of two experiments. The standard clinical criteria to define the exacerbation are described in [42]. A total of three MRI scans were performed at three different time points, baseline scan (before vaccination), after the depletion of MBP-reactive T cells (scan 2) and 1–3 years after vaccination (scan 3), respectively. The method for semiquantitative measurement of MRI brain lesions is described in [43]



tion and at the end of the trial (2–3 years after vaccination). In parallel two MRI scans (before and at the end of the trial) were performed in a similar manner for each control subject. Our semiquantitative analysis of the MRI scans revealed that in five out of eight vaccinated patients, the total lesion score of the two MRI scans (taken shortly after vaccination and approx. 2 years after vaccination) did not differ significantly from respective baseline values, representing merely a 3.6% lesion increase. In contrast, among the control subjects the brain lesions progressed over the same period of time. The total Δ MRI lesion score of the control group was increased by 39.5% in the scans taken at the end of the trial compared to the baseline scans. The stabilization of the brain lesions seen in some vaccinated patients may represent a significant improvement, considering that in an unintervened course the lesions deteriorate generally by at least 10% on a yearly basis as seen in the control subjects. Figure 2 summarizes the the clinical results in relation to the changes in the frequency of circulating MBP-reactive T cells in nine vaccinated patients.

Taken together the results of this clinical trial suggest a moderate clinical improvement in some relapsing-remitting MS patients vaccinated with irradiated MBP-reactive T cells, with respect to a reduced rate of exacerbation and relative stabilization in the disease scores (EDSS) and the brain lesion pathology. Although the limitation associated with the small number of subjects tested in this open-label trial prohibits to draw a firm conclusion regarding the treatment efficacy, the results encourage further double-blind clinical trials involving more MS patients at various stages of the disease.

Remarks and future considerations

There are a number of intriging findings that have emerged from this study. First, it has confirmed that clonally expanded MBP-reactive T cells in MS represent a dominant TCR repertoire and depletion of this population(s) eradicates the total T cell responses to MBP. The vaccinated subjects maintained sufficient immunity towards the immunizing MBP-reactive T cell clones over a period of 1–2.5 years. This immunity correlates with the undetectable level of circulating MBP-reactive T cells in the majority of the recipients. As illustrated in Fig. 2, in some vaccinated MS patients, however, MBP-reactive T cells reappeared in circulation 18–21 months after vaccination, and the reappearing clones represented different clonal origins that were not detected before vaccination, suggesting clonal shift of MBP-reactive T cells in these patients [40]. It is of interest to note that these three patients underwent a significant clinical deterioration and the worsening of the MRI brain lesions at the time when circulating MBP-reactive T cells reappeared. The results suggest that in these cases the three concurrent events, namely the exacerbation, worsening of MRI lesions, and reappearance of MBP-reactive T-cells, may be related to a common pathological change(s) attributable to a triggering episode that induced the clinical relapses. This observation lends further support to the potential role of MBP-reactive T cells in the pathogenesis of MS. It is likely that when the dominant clones were depleted after immunization, the previously cryptic clones emerged in response to a persistent in vivo "priming event" responsible for in vivo activation of MBP-reactive T cells, such as breakdown MBP products or a cross-reactive antigen. Alternatively, these MBP-reactive T cell clones may be activated and further expanded by a new "priming event" associated with clinical exacerbation recorded in two of the three subjects at the time when these T cells reappeared in circulation. On the other hand, this finding needs to be taken into consideration in our attempts to improve the current T cell vaccination protocol for future clinical trials. In cases where the TCR repertoire of a given antigen specificity represents oligoclonal origins, it is preferable to use a "cocktail" vaccine preparation containing various clones characteristic of each individual clonal origin. Similar consideration may also apply to other TCR-based specific therapies where T cells are selectively depleted or suppressed based on their clonal structural characteristics.

Our recent study further demonstrated that the regulation of MBP-reactive T cells induced by T cell vaccination is clonotype-specific [40]. The conclusion is based on a series of experiments carried out to study the recognition pattern of the anticlonotypic T cell responses and resulting anticlonotypic T cell lines. (a) The clonotypic recognition was reflected by the T cell responses that were directed specifically at the immunizing clones but not the reappearing clones and control T cells. This suggests that the clonotypic regulatory network is enhanced specifically to regulate the clone(s) used for vaccination. The clonotype-specific recognition enables the regulatory network to distinguish the immunizing T cell clones from other unrelated T cells. (b) As we demonstrated recently [40], when more than two structurally unrelated MBP-reactive T cell clones are present in the T cell repertoire, vaccination with one T cell clone induces an anticlonotypic response that recognizes selectively the immunizing clone and does not affect the other clone(s). (c) The anticlonotypic T cell lines could only be generated by and respond to repeated stimulation with the immunizing T cell clones and not the reappearing clones or PHA-induced nonspecific T cells.

Another important aspect of this study is related to the molecular target of the cytolytic anticlonotypic T cells induced by T cell vaccination. Our study provides some preliminary evidence suggesting that hypervariable regions of the V genes may contribute to the observed clonotypic interactions. The results suggest that both the CDR3 region unique for individual clones and an additional V gene region encoding for a less variable sequence may be involved in the clonotypic recognition. First, the observation that the anticlonotypic T cell lines recognize specifically the immunizing clone but not the other autologous MBP-reactive clones with different clonal origin(s) argues for a recognition pattern of the

CDR3 region, which is specifically directed at a unique V gene junctional sequence of the immunizing clones. On the other hand, our data suggest that although the CDR3 region may be dominant in the observed cytolytic clonotypic recognition, it is not exclusive. A less variable TCR region may also be involved in interacting with the anticlonotypic T cells. For example, we demonstrated that a cytolytic anticlonotypic T cell clone isolated from a vaccinated patient recognized a nonimmunizing MBP-reactive T cell clone which shared the identical Vα chain but not the Vβ with the original immunizing clone and both target T cell clones had unrelated V-D-J and V-J junctional sequence pattern [40].

The information regarding the molecular target of the cytolytic anticlonotypic T cells is essential to our attempt to improve and simplify the current T cell vaccination protocol. At this time the current data are not sufficient to predict whether T cell vaccination will remain a "personalized" treatment, and whether it may be suited for a category of patients whose targeted autoreactive T cells share a common TCR structural feature. A more generalized form of T cell vaccination may depend on its simplified version that takes the advantage of using synthetic peptides incorporating common sequence characteristics of the target TCR in combination with limited MHC class I motifs. Our on-going sequence analysis and the experiments using autologous and MHC-matched transfectants expressing various candidate TCR region sequences will provide some answers to this issue.

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